Acid-base homeostasis of blood and pH of abomasum in calves fed non-acidified and acidified milk replacer

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ABSTRACT: Experiments were conducted on calves divided into three groups, 6 animals in each, to determine the influence of the intake of non-acidified and acidified milk replacer on the level of acid-base homeostasis in venous blood. The milk replacer was supplied at a dose of 700 g dry matter in 8 l of the liquid in two feedings. The milk replacer was acidified by adding formic acid to ensure the concentration of 0.2% in milk replacer. Venous blood was collected before feeding and 1, 2, 4, and 6 hours after feeding. The samples were analyzed for blood pH, actual bicarbonate HCO_3^-(mmol/l), base excess BE (mmol/l), partial pressure of carbon dioxide pCO_2 (kPa) and partial pressure of oxygen pO_2 (kPa). Significant changes were observed in calves fed acidified milk of albumin type. The mean daily values of acid-base parameters in these calves were significantly lower in comparison with animals fed non-acidified milk replacer and reached the following levels: pH 7.343 ± 0.032 ($P < 0.05$), HCO_3 24.49 ± 2.13 mmol/l ($P < 0.01$), BE 1.11 ± 1.97 mmol/l ($P < 0.001$). A similar tendency but of a more pronounced decrease in values was recorded in the group of calves fed acidified milk replacer of casein type: pH 7.312 ± 0.022 ($P < 0.01$), HCO_3^- 21.73 ± 0.75 mmol/l ($P < 0.001$), BE 96 ± 0.86 mmol/l ($P < 0.001$). In relation to the time after feeding the group of calves fed non-acidified milk replacer showed a rising tendency in the level of metabolic components (HCO_3^-), compensated by respiratory regulating mechanisms (rise in pCO_2) conducive to the maintenance of optimum blood pH level. The group of calves fed acidified milk replacer (formic acid 2 ml/l) of the albumin type showed metabolic acidosis with subsequent gradual adjustment and compensation by means of metabolic (HCO_3^-) rather than respiratory regulation mechanisms. The calves fed acidified casein type milk replacer displayed metabolic acidosis with insufficient metabolic regulation and more intensive respiratory compensation (decrease in pCO_2). Concurrent investigations of the abomasum acidity and blood acid-base homeostasis reflected the joint action of both the acidifying effect of formic acid and significantly lower production of bicarbonate (HCO_3^-) related to the intake of acidified milk and the tendency to the development of metabolic acidosis.

Keywords: calves; milk feeding; formic acid; acid-base balance; pH of abomasums

The acid-base balance of blood in suckling calves is influenced by the level of hypoxia and hypercapnia in the peripartal period resulting in respiratory-metabolic acidosis dependent on difficulties of the parturition and influence of stress (Szenci et al., 1989). The acid-base profile in calves with respiratory syndrome developed to various degrees tends towards respiratory acidosis (Janeczek and Kolacz, 1988).

Due to nutrition, the acid-base balance is influenced significantly by the quantity and quality of milk feed mixtures and substitute plant or animal proteins and fats in milk replacer in relation to the secretory capacity of abomasum (Reece and Wahlstrom, 1972). According to the character of proteins with respect to their buffering capacity, the secretion of HCl is regulated towards optimum proteolytic digestion. An increasing secretion of HCl at a higher buffering capacity of proteins results in alkalization of the internal environment (Hill, 1970). The abundance of fat, particularly milk fat with a high proportion of short-chain fatty acids after hydrolysis by pre-gastric esterase, also contributes to acidification of the abomasum (Otterby et al., 1964).

The mineral composition of milk replacer as regards the cation-anion balance of mineral salts influences both the Ca metabolism and the acid-base balance of blood and decreases pH, pCO_2, PO_2, HCO_3^- and ABE at the anionic type of minerals (Jackson and Hemken, 1994).

Exogenous supply of a high quantity of organic acids or highly soluble inorganic acids and their absorption through the digestive system causes a reduction in alkaline reserves in the circulating blood with a tendency to acidify the internal environment (Eidelsburger et al., 1992).

The aim of the study was to define the daily dynamics of the acid-base status in relation to time after feeding the calves with acidified and non-acidified milk replacer. Another aim was to observe the changes in abomasal pH in relation to the level of blood acid-base values related to post-feeding time.

MATERIAL AND METHODS

Animals

In the first experiment we investigated the influence of the feeding of milk replacer on the level of blood acid-base homeostasis in 18 calves under experimental conditions. At the age of 2 weeks the calves weighing 40–45 kg were divided into three groups and fed 8 l of milk replacer per head/day in 2 feedings.

Diets and feeding

The calves in the 1st group were fed non-acidified (pH 6.4–6.6) warm milk replacer of casein type. The milk replacer was reconstituted with water (90 g/l) and supplied in a bucket at a temperature of about 40°C.The calves in the 2nd group were fed acidified cold (16–18°C) milk replacer of the same type. Calves in the 3rd group received acidified cold milk replacer of albumin type based on dried whey and soya proteins. Conventional milk replacer of albumin type contained: dried whey 55%, soya protein concentrate 25%, vegetable fat 18%, vitamin and mineral premix 2.0% (CP 20.0%, lysine 1.9%, fat 18.0%, 16.5 MJ ME, Ca 0.9%, P 0.7%). Milk replacer of casein type based on dried skim milk 32%, dried whey 28%, whey protein concentrate 20% and vegetable fat 18%, vitamin and mineral premix 2.0%, contained CP 22.0%, fat 17%, 15.8 MJ ME, Ca 0.8%, P 0.6%. The milk replacer was acidified with concentrated formic acid (85%) at a dose of 2 ml/l, which ensured that the pH value of milk replacer ranged between 4.2 and 4.6. The calves were kept in groups at temperatures between 16° and 18°C and had free access to water and calf starter mix. Commercial pelleted calf starter (CP 17.0%, fat 3.0%, NEL 7.1 MJ, ash 4.9%, Ca 0.9%, P 0.8%) was offered once daily for *ad libitum* consumption.

Blood collection

Blood samples were collected from the jugular vein in the 4th week of life immediately before feeding and 1, 2, 4 and 6 hours after feeding during two days. The venous blood samples collected into heparinised glass capillaries were stored on ice and they were analyzed within one hour of collection by an ABL analyzer (Radiometer Copenhagen).

To assess the acid-base balance we determined the level of blood pH (log-molc), partial pressure of carbon dioxide pCO_2 (kPa), partial pressure of oxygen pO_2 (kPa), bicarbonate HCO_3^- (mmol/l) and base excess BE (mmol/l).

In the second experiment four calves with abomasal fistulae were analyzed in parallel for the level of the abomasum acidity and blood acid-base values in relation to time after feeding.

Statistical analysis of results

Results were processed by computer software GraphPad Prism Vers. 4 to obtain means and standard deviations in relation to feeding time and the type of milk replacer. The significance of nutritional effect and the time of feeding on the studied parameters were analyzed by *F*-test to compare variances and by unpaired *t*-test. Differences between the decreases in pH in calf abomasum content samples after feeding were checked by paired *t*-test.

RESULTS

Dynamics of the level of acid-base parameters of venous blood in calves fed non-acidified and acidified milk replacer

Diurnal means of blood acid-base values in calves fed non-acidified and acidified milk replacer of casein and albumin type are summarized in Table 1.

Table 1. The diurnal means of blood acid-base values in calves in relation to nutrition ($x \pm sd$)

Parameters	Reference values*	Non-acidified milk replacer	Acidified milk replacer	
			casein type	albumin type
pH	7.23 – 7.41	7.379 ± 0.029	7.312 ± 0.022	7.343 ± 0.032
HCO_3^-(mmol/l)	26.7 – 33.7	28.02 ± 1.18	21.73 ± 0.75	24.49 ± 2.13
BE (mmol/l)	–0.5 ± 35	2.54 ± 1.67	–3.96 ± 0.86	–1.11 ± 1.97
pCO_2 (kPa)	5.8 – 7.4	6.55 ± 0.77	5.66 ± 0.39	6.13 ± 0.60
pO_2 (kPa)	4.5 – 6.0	4.17 ± 1.4	4.36 ± 0.56	4.40 ± 0.79

*Summary from Table 6

Table 2. The values of acid-base balance ($x \pm sd$) in calves (n-6) fed non-acidified milk replacer

Parameters	Time after feeding in hours				
	0	1	2	4	6
pH (log molc)	7.384 ± 0.029	7.394 ± 0.035	7.380 ± 0.031	7.376 ± 0.005	7.377 ± 0.009
pCO_2 (kPa)	6.29 ± 0.52	6.05 ± 0.45	6.14 ± 0.32	6.92 ± 1.00	7.44 ± 0.28
HCO_3^-(mmol/l)	27.7 ± 1.14	27.3 ± 0.36	26.8 ± 1.93	27.4 ± 0.41	31.0 ± 0.57
BE (mmol/l)	2.27 ± 1.03	2.3 ± 0.86	1.67 ± 2.04	1.57 ± 0.71	4.87 ± 0.89
pO_2 (kPa)	5.03 ± 0.20	4.19 ± 0.59	4.83 ± 1.05	3.49 ± 0.79	2.93 ± 0.29
SBC (mmol/l)	25.9 ± 0.74	25.9 ± 0.80	25.4 ± 1.64	25.1 ± 0.97	27.9 ± 0.84

Acidity

pH of venous blood reached the highest value in calves fed warm non-acidified milk replacer. Lower values ($P < 0.05$) were found out when feeding acidified milk replacer of albumin type, and significantly lower values ($P < 0.01$) when feeding acidified milk replacer of casein type. The highest level of actual bicarbonate (HCO_3^-) was determined in calves fed non-acidified milk replacer. Calves fed acidified milk showed a lower ($P < 0.01$) level of HCO_3^- when fed the albumin type, and a significantly lower ($P < 0.001$) level when fed the casein type of milk replacer. Similarly, BE showed a decreasing trend from 2.54 ± 1.67 mmol/l for non-acidified milk replacer and significantly lower values ($P < 0.001$) for acidified milk replacer: albumin type –1.11 ± 1.97 mmol/l, casein type –3.96 ± 0.86 mmol/l. The partial pressure of blood gases, carbon dioxide (pCO_2) and oxygen (pO_2) were not influenced by nutrition significantly.

The dynamics of the level of acid-base parameters in relation to the time after feeding is summarized in Tables 2–4.

The calves fed non-acidified milk replacer and investigated in relation to the time after feeding (Table 2) showed small fluctuations in venous blood acidity which remained close to the middle value of the reference range in all collections. Six hours after feeding the bicarbonate level approached the upper level of the diurnal fluctuation and the increase was significant ($P < 0.01$). A similar tendency was observed in the values of base excess which increased significantly ($P < 0.01$) within 6 hours after feeding. Partial pressure, pCO_2, increased in samples taken at hour 4 and 6 after feeding and showed a tendency of hypercapnia.

The venous blood collected from calves fed non-acidified milk replacer indicated an increase in the level of metabolic regulatory mechanisms (HCO_3^-) with elements of respiratory compensation (decrease in pO_2 and increase in pCO_2) of the inner environment within the acid-base balance homeostasis.

The calves fed albumin type acidified milk replacer and observed in relation to the time after feeding (Table 3) exhibited lower acidity with a decrease in pH in the first hour after feeding and consecutive marginal values slightly below the lower limit of the reference range. The dynamics of HCO_3^-, BE and pCO_2 showed a significant increase starting at hour 2 after feeding up to hour 6 ($P < 0.001$), when the mean values reached the reference range.

The acid-base parameters of venous blood in this group indicated metabolic acidosis with subsequent

Table 3. The values of acid-base balance ($x \pm sd$) in calves (n-6) fed acidified milk replacer, albumin-type

Parameters	Time after feeding in hours				
	0	1	2	4	6
pH (log molc)	7.351 ± 0.039	7.325 ± 0.041	7.346 ± 0.021	7.349 ± 0.02	7.345 ± 0.024
pCO_2 (kPa)	5.87 ± 0.75	6.19 ± 0.53	5.71 ± 0.23	6.11 ± 0.39	6.76 ± 0.35
HCO_3^- (mmol/l)	23.87 ± 2.87	23.65 ± 1.29	22.97 ± 0.72	24.8 ± 0.82	27.2 ± 0.89
BE (mmol/l)	–1.53 ± 2.6	–2.15 ± 1.56	–2.3 ± 0.89	–0.75 ± 0.69	1.32 ± 0.92
pO_2 (kPa)	5.02 ± 0.37	4.32 ± 0.28	5.2 ± 0.87	3.87 ± 0.91	3.91 ± 0.43
SBC (mmol/l)	22.7 ± 2.18	21.95 ± 2.0	22.1 ± 0.75	23.1 ± 0.39	24.9 ± 0.81

Table 4. The values of acid-base balance ($x \pm sd$) in calves (n-6) fed acidified milk replacer, casein-type

Parameters	Time after feeding in hours				
	0	1	2	4	6
pH (log molc)	7.312 ± 0.031	7.271 ± 0.022	7.310 ± 0.26	7.321 ± 0.026	7.312 ± 0.014
pCO_2 (kPa)	6.45 ± 0.72	6.132 ± 0.489	5.58 ± 0.274	5.71 ± 0.115	5.93 ± 0.425
HCO_3^-(mmol/l)	22.6 ± 0.86	20.97 ± 1.33	20.67 ± 1.29	21.6 ± 1.34	22.1 ± 1.33
ABE (mmol/l)	–3.0 ± 0.86	–4.97 ± 1.20	–4.75 ± 1.51	–3.70 ± 1.28	–3.4 ± 1.29
pO_2 (kPa)	4.19 ± 0.48	5.03 ± 1.38	4.29 ± 0.40	3.68 ± 0.38	3.55 ± 1.08

compensation by metabolic (HCO_3^-, ABE) and low respiratory regulatory mechanisms.

The observation of calves fed casein-type acidified milk replacer showed that the mean values of pH, pCO_2 and BE (Table 4) in blood samples collected in relation to the time after feeding were lower and increased significantly up to hour 6 after feeding.

The acid-base status indicated continuous acidosis with insufficient compensation by metabolic regulatory mechanisms and more intensive application of respiratory compensation and decrease in the level of pCO_2.

Dynamics of abomasal pH in relation to the level of acid-base homeostasis of venous blood in calves

Abomasal acidity in calves

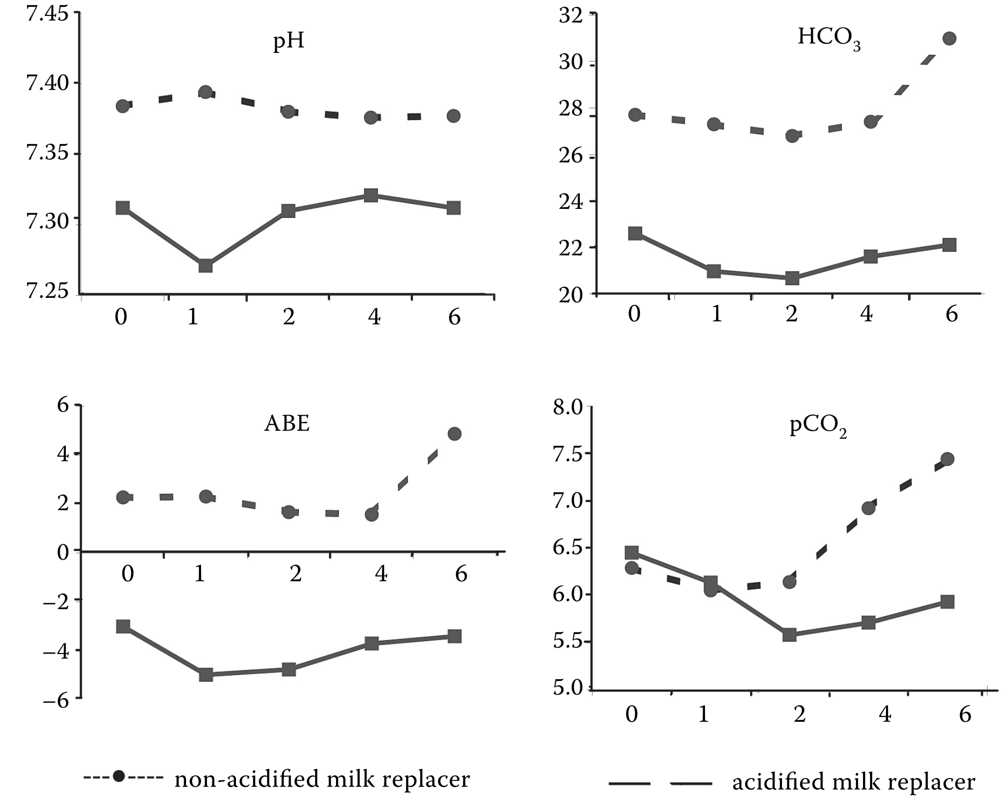
The substantial acidifying effect of formic acid added at a dose of 2 ml per 1 l of milk maintains the acidified milk replacer in the pH range 4.2–4.4 in comparison with pH 6.7–6.9 of non-acidified milk replacer. The intake of milk replacer of the same nutritional composition with and without supple-

Table 5. The dynamics of abomasal pH in calves ($x \pm sd$)

Hour of sampling	Non-acidified milk replacer	Acidified milk replacer	Significant difference
0	2.95 ± 0.4	3.29 ± 0.5	NS
1/4	5.74 ± 0.5	4.68 ± 0.3	$P < 0.05$
1	5.58 ± 0.2^{NS}	4.25 ± 0.3^{a}	$P < 0.001$
2	5.25 ± 0.4^{a}	4.08 ± 0.3^{b}	$P < 0.001$
3	5.00 ± 0.5^{b}	3.40 ± 0.3^{b}	$P < 0.001$
4	4.68 ± 0.3^{b}	2.68 ± 0.2^{b}	$P < 0.001$
5	4.21 ± 0.3^{b}	2.54 ± 0.2^{b}	$P < 0.001$
6	3.91 ± 0.5^{b}	1.87 ± 0.3^{b}	$P < 0.001$

[a,b]Statistical evaluation of a decrease in pH in the calf abomasum content sampled 15 min after feeding: (paired t-test, $^{a}P < 0.05$; $^{b}P < 0.001$, NS – not significant

F- test to compare variances – NS



Time after feeding –0, 1, 2, 4, 6 hour

Figure 1. The dynamics of acid-base values in venous blood in calves

mentation of formic acid affected the abomasal acidity in calves differently in relation to the time after feeding (Table 5). Within 15 min after feeding, the acidity of the abomasal content increased significantly ($P < 0.001$) from pH 2.95 ± 0.4 and 3.29 ± 0.5 to pH 5.74 ± 0.5 and 4.68 ± 0.3 when feeding non-acidified and acidified milk replacer, resp. In all samples taken in relation to the time after feeding, the abomasal pH was significantly lower in calves fed acidified milk in comparison with those fed non-acidified milk replacer. The abomasal pH decreased below 4.0 in 2 hours and 5 hours after feeding when supplying acidified and non-acidified milk replacer, resp. Sour milk replacer together with secreted HCl result in optimum abomasal acidity which supports the activity of proteolytic enzymes.

The dynamics of the acid-base values in venous blood in relation to the time after feeding non-acidified and acidified milk replacer of identical composition is summarized in Figure 1. The mean values of the acidity of venous blood were significantly lower ($P < 0.01$) in calves fed acidified milk at all collection intervals. A different tendency in the dynamics of acidity was observed only one hour after feeding. The calves that received non-acidified milk replacer showed a rising tendency and the highest level of blood pH, while those fed acidified milk showed a significant decrease one hour after feeding and the lowest mean pH level of venous blood within the daily dynamics.

The dynamics of mean bicarbonate levels (HCO_3^-) showed a decrease up to two hours after feeding in both groups of calves. The consecutive rise, culminating 6 hours after feeding, was more pronounced in calves fed non-acidified milk replacer in which the HCO_3^- content exceeded the initial level before feeding. On the other hand, bicarbonate in calves fed the acidified milk diet increased gradually and in 6 hours after feeding it failed to reach the initial level. The bicarbonate (HCO_3^-) level in all samples collected from the group of calves fed non-acidified milk replacer fluctuated within the physiology range while that in calves fed acidified milk was significantly lower ($P < 0.001$) in all collected samples.

The partial pressure of CO_2 (pCO_2) in both groups of calves after feeding showed a decreasing tendency. Due to a significant rise ($P < 0.001$) in this parameter in calves fed non-acidified milk replacer, it reached the upper reference limit within 6 hours after feeding. On the other hand, pCO_2 in calves fed acidified milk replacer fluctuated after feeding close to the lower physiological limit.

Excess bases (BE) in calves fed non-acidified milk replacer remained more or less constant up to hours 4 after feeding and then the mean value of this parameter increased to the level close to the upper physiological limit. The observation after feeding showed that calves fed acidified milk exhibited BE values below the lower negative limit of excess bases.

DISCUSSION

Our observations of the acid-base homeostasis of calf blood in the liquid-nutrition phase in relation to the feeding regimen were based on the analysis of venous blood with the aim to minimise the respiratory compensation of the acid-base state of the internal environment. Investigations of the acid-base parameters in the arterial and venous blood of calves conducted by Slanina et al. (1992) showed significantly higher values ($P < 0.001$) of pCO_2 and pO_2 in arterial blood and higher values of HCO_3^- , pCO_2 and ABE in venous blood.

Comparison of our results with the mean values in venous blood calculated on the basis of available references is presented in Table 6. The authors referred to in this table recommended different reference ranges for respective parameters but the mean values of these parameters were very similar.

The feeding of acidified milk replacer influenced the acid-base homeostasis in calves with a tendency to acidosis. This effect is based on the direct acidifying effect of formic acid resulting in decreased acidity of blood. The results allowed us to assume also an indirect influence on the acid-base values through the regulation of secretory capacity of the abomasum by the acidified milk replacer.

The calves fed non-acidified milk replacer showed the balanced dynamics of pH and a significant rise in HCO_3^- , pCO_2 and ABE in venous blood. This increase was in agreement with the decrease in abomasal pH. The release of hydrogen ions (H^+) for the production of HCl by parietal cells of the abomasum is associated with the parallel production of bicarbonate ions (HCO_3^-) which penetrate into the blood stream (Hill, 1970). Thus, through the abomasum secretory capacity the type of milk replacer affects the concentration of bicarbonate (HCO_3^-) in the blood in dependence on the amount of secreted HCl needed for a decrease in abomasal pH to the level optimal for abomasal digestion.

While feeding calves with milk feed mixture, Reece (1980) found significantly higher values of pH, HCO_3^- and ABE compared to feeding whole

Table 6. Recommended reference values of the acid-base balance of calf venous blood

Author	pH (log molc)	HCO_3^- (mmol/l)	pCO_2 (kPa)
Janeczek and Kolacz (1988)	7.39 – 7.41	28.51 – 36.39	6.0 – 8.9
	7.398	32.45	7.45
Vestweberg et al. (1977)	7.35 – 7.47	21.9 – 39.4	4.93 – 7.13
	30.65	6.03	7.410
Schotman (1971)	7.36 – 7.43	27.3 – 27.6	4.86 – 6.2
	7.395	27.45	5.53
Slanina et al. (1992)	7.35 – 7.38	29.3 – 31.34	7.24 – 7.46
	7.362	30.32	7.35
Rosemberger (1990)	7.23 – 7.37	–	5.8 – 7.2
	7.300		6.5
Nagy et al. (2003)	7.35 – 7.38	24.2 – 27.4	5.7 – 6.5
	7.363	25.8	6.1
Summary average	7.23 – 7.41	26.75 – 33.72	5.77 – 7.38

milk. The acid-base state indicated metabolic alkalosis with respiratory compensation culminating in the 4^{th} week and gradual adjustment with age. The degree of alkalosis was significantly higher in the milk mixture compared to whole milk (Reece and Wahlstrom, 1972).

The feeding of acidified milk replacer affected considerably both the abomasal pH and the dynamics of the acid-base blood markers. To achieve our aim regarding the feeding regimen, we used two types of acidified milk replacer differing in the type of proteins. The acidifying effect was more pronounced in the casein type of milk replacer compared to the albumin one (whey and extracted soya protein).

The type of proteins in a liquid feed affects its buffering capability related to its isoelectric point and therefore also its influence on the acid-base balance in relation to the time after feeding. While the isoelectric point of casein is 4.5, in albumins of whey and soya it is 5.3. The protein with higher isoelectric point acts as a more effective buffer.

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Received: 2006–07–18
Accepted after corrections: 2007–02–07

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